THE THERMOTROPIC BEHAVIOR OF DIPALMITOYL PHOSPHATIDYLCHOLINE BILAYERS

A Fourier Transform Infrared Study of Specifically Labeled Lipids

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ABSTRACT Fourier transform infrared spectroscopy has been used to study the thermotropic behavior of hydrated multibilayers of specifically deuterated derivatives of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. It is shown that throughout the gel phase there is little or no conformational disorder introduced into the acyl chains. The pretransition effect is greatest in the central segments of the acyl chains, demonstrating that the interchain interactions are more pronounced in this region than in the center of the bilayer and suggesting the presence, in the gel phase, of a "plateau" in the strength of the interchain interactions. As the temperature is reduced, the rate of rotation of the terminal methyl group decreases steadily; below 0°C the conformation is rigid on the infrared time scale.

INTRODUCTION

When hydrated, 1,2-dipalmitoyl-*sn*-glycero-phosphatidylcholine forms multibilayers which exhibit complex structural changes in the temperature range from −60° to +70°C. The chief features of the thermotropic behaviour are: a transition at 41.5°C (T_m) from a gel phase in which the acyl chains are fully extended to a liquid crystalline phase in which there is a large population of *gauche* conformers; a pretransition event at about 36°C (T_{pre}), where there is an abrupt change in the unit cell in which the acyl chains are packed; and a continuous change in the gel phase packing as the temperature is reduced below T_{pre}.

Deuterium NMR studies of a series of specifically deuterated dipalmitoyl phosphatidyl cholines have provided detailed order parameter profiles of the liquid crystalline phase and have led to the concept of an order "plateau" in this phase (1). Progress in the understanding of the gel phase structure has resulted principally from studies using techniques that monitor simultaneously the properties of all the methylene groups. It has been demonstrated that the pretransition results in a change in the unit cell in which the acyl chains are packed (2), from hexagonal above T_{pre} to an extremely loose orthorhombic subcell below T_{pre} (3). A further reduction in temperature results in a decrease in the amplitudes of the torsional and

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librational motions about the long axes of the acyl chains (4–6), and increases the rigidity of the orthorhombic subcell (6).

Despite the obvious attraction of a study of the gel phase spectra of a series of specifically deuterated compounds, only one laser Raman study of deuterated dimyristoyl phosphatidylcholines has been reported (7). Owing to the signal-to-noise ratio, however, the study was restricted to changes in bandwidth at T_m, and hence no detailed data are available on the spectral changes in the gel phase and the specific changes at T_{pre}.

Herein we report on changes observed in the C—D stretching region of the infrared spectra of a series of specifically deuterated dipalmitoyl phosphatidylcholines (DPPC).[1] By studying the infrared absorption spectra of CD_2 and CD_3 groups located at the 2', 3', 7', 8', 13', and 16' positions of the *sn*-2 palmitoyl chain, we obtain spectral profiles of the hydrophobic region of the lipid bilayer and relate the data to the structure of the bilayer in the temperature range 5–55°C.

EXPERIMENTAL

Materials and Sample Preparation

Details of the synthesis and purification of DPPC-2'-d_2^1, DPPC-3'-d_2, DPPC-7',8'-d_4, DPPC-13'-d_2 and DPPC-16'-d_3 have been reported (8). Fully hydrated samples in 25 or 50 micrometer-thick CaF_2 cells, depending on the strength of the C—D stretching bands, were prepared according to techniques described in detail in reference 9. The purities of the materials were checked by thin-layer chromatography, which in all cases showed only a single spot. A second criterion was the thermotropic behavior as monitored via the CH_2 scissoring and wagging bands of the infrared spectra. For each sample pretransitions and sharp, well-defined main transitions were observed, similar to those found in a study of perhydro DPPC (3). This is particularly relevant to the spectra of DPPC-2'-d_2 and DPPC-3'-d_2. In these samples, particularly the former, T_m is broad when monitored via the CD_2 stretching bands.

Operating Conditions

Spectra were recorded on a Digilab FTS-15 Fourier transform infrared (FT-IR) spectrometer equipped with a 2-mm-diam InSb detector (Infrared Associates, New Brunswick, N. J.) and a 2,500 cm^{-1} low-pass optical filter. Typically, 500 interferograms, recorded as an optical velocity of 1.2 cm s^{-1} with a maximum optical retardation of 1 cm, were co-added, apodized with a triangular function, and Fourier-transformed with one level of zero filling resulting in a spectral resolution of 0.9 cm^{-1}.

Temperature regulation in the range 0–55°C was achieved by circulation of a thermostated ethanol-water mixture through a hollow cell mount, which provides a temperature stability throughout the sample of better than ±0.1°C. A thermocouple was mounted directly on the cell window and temperatures were recorded by a Newport digital pyrometer (Newport Laboratories Inc., Santa Ana, Calif.) equipped with a printer. All results were reproduced four times in discrete experiments. In each case during a sequence of measurements the sample and spectrometer were untouched, the only operating parameter varied being the temperature. The complete sequence of obtaining a spectrum, printing and incrementing the temperature, waiting for equilibration, and recording the next spectrum is under the control of the spectrometer computer. Typically, the spectral collection took 10 min, and 20 min was allowed for increasing and equilibrating the sample temperature.

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[1] *Abbreviations:* DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPC-2'-d_2, 1-palmitoyl-2-(2'-dideutero)-palmitoyl-*sn*-glycero-3-phosphocholine; DPPC-3'-d_2, 1-palmitoyl-2-(3'-dideutero)-palmitoyl-*sn*-glycero-3-phosphocholine; DPPC-7',8'-d_4, 1-palmitoyl-2-(7',8'-tetradeutero)-palmitoyl-*sn*-glycero-3-phosphocholine; DPPC-13'-d_2, 1-palmitoyl-2-(13'-dideutero)-palmitoyl-*sn*-glycero-3-phosphocholine; DPPC-16'-d_3, 1-palmitoyl-2-(16'-trideutero)-palmitoyl-*sn*-glycero-3-phosphocholine; DPPC-d_{62}, 1,2 diperdeuteriopalmitoyl-*sn*-glycero-3-phosphocholine.

Data Processing and Accuracy

$\Delta A/\Delta T$ values were obtained directly from difference spectra generated by successive subtraction of a series of absorbance spectra recorded at different temperatures (9).[2]

Frequencies and bandwidths were determined from the spectra of the phosphocholines after the broad "water association" band centered at about 2,150 cm^{-1} was removed by spectral subtraction. As this band also varies with temperature, the spectrum of water was recorded at 2°C intervals over the range 1–55°C using the same spectral conditions as outlined above. This enables both the pathlength and the temperature to be matched when the subtraction is carried out.

Frequencies were measured by determining the center-of-area (10) of the topmost five data points of the peak[3], and checked by comparison with center-of-area values determined from three and seven points. This provides a precise method for determining the frequency of the maximum position of an absorbance-band contour. The wavenumber scale of an FT-IR spectrometer using HeNe fringes to determine the optical path difference is reproducible to within 0.005 cm^{-1}. Thus, as the spectral resolution is substantially less than the halfwidth of the bands (10 cm^{-1} or greater), and as the only parameter varied in a given temperature run is the temperature, the only significant factor affecting the precision to which shifts in frequency can be monitored is the signal-to-noise ratio. With the signal-to-noise ratios employed in this study, and the use of the center of area, temperature-induced shifts as small as 0.05 cm^{-1} were measured.

In principle, incorrect subtraction of the water background might affect the measured frequency values. We found, however, no effect even when the scaling factor was varied by ±50%. We attribute this to the major difference in bandwidths of the lipid and water spectra. The lipid bands have halfwidths in the range 10–40 cm^{-1}, whereas the water band has a halfwidth of about 500 cm^{-1}. Thus, incorrect subtraction of the water band has only the effect of introducing a slope to the base line of the spectrum with no resultant change in the frequency of the absorbance maxima.

Bandwidths were measured by digitally subtracting a linearly interpolated base line from the spectrum, and then computing the width at halfheight of the resultant band contour. This approach yields an accurate and consistent way of monitoring bandwidths. However, the absolute values are not the true halfwidths of the isolated bands, but only a first approximation to them. This precludes a comparison of small differences in bandwidth as a function of position of the CD_2 or CD_3 group in the acyl chain. Furthermore, as there are major changes in the bandshapes at T_m, a comparison of the relative magnitudes of the changes in bandwidths at T_m is not possible.

RESULTS AND DISCUSSION

Assignment and General Temperature Dependence

The carbon-deuterium stretching region of the vibrational spectra of compounds containing specifically deuterated acyl chains generally consists of two strong bands near 2,200 and 2,100 cm^{-1} (11). In all cases the band near 2,200 cm^{-1} is assigned to the asymmetric (CD_3) or antisymmetric (CD_2) stretching mode. When a single band is observed near 2,100 cm^{-1} it is assigned to the symmetric stretching mode. Frequently, an additional weak band is observed near 2,130 cm^{-1}, and these two bands, ~2,130 and ~2,100 cm^{-1}, are assigned to the symmetric stretching fundamental in Fermi resonance with the first overtone of the CD_2 or CD_3 bending mode. In any case, the behavior of these bands may be more complex than that of the asymmetric band, as spectral changes may result from variations in the Fermi

[2]In our earlier publications (3, 9) we used the notation $\Delta A/°C$ for this parameter, but in the present study we use the more correct $\Delta A/\Delta T$.

[3]With the resolution used, and using one level of zero filling in the transform, the encoding interval of the data is about 0.5 cm^{-1}; the values computed are hence centers of area for the topmost 2.5 cm^{-1} wide segments of the peaks.

resonance interaction, as well as from changes in the acyl chain conformations and freedom of motion. Consequently, in the following sections we have largely restricted the discussion to the asymmetric stretching band near 2,200 cm^{-1}. Nonetheless, the experimental observation is that, except for the case of DPPC-2′-d_2, the changes observed in the two stronger bands, near 2,200 and 2,100 cm^{-1}, are similar in magnitude and direction. This point is illustrated in Table I, which summarizes the changes in frequency of these bands during the main transition. Also included are data taken from the spectra of anhydrous films of the specifically deuterated phospolipids, and data taken from the solid phase spectra of a series of specifically deuterated palmitic acids.

From an examination of the results obtained with the hydrated phospholipids it is evident that the main melting transition results in changes in both the asymmetric and symmetric C—D stretching bands of most of the compounds studied. It is also evident that the magnitudes of the changes in the spectra of DPPC-16′-d_3 and DPPC-2′-d_2 are smaller than those in the spectra of the other compounds, and thus we have subdivided the detailed presentation of the results into three sections according to the position and type of substitution.

DPPC-2′-d_2

The CD_2 group of DPPC-2′-d_2 exhibits spectral properties substantially different from those of DPPC-3′-d_2, DPPC-7′,8′-d_4 and DPPC-13′-d_2: The CD_2 stretching bands are extremely weak, the antisymmetric band contains two principal components separated by ~3 cm^{-1}, and the frequency of this band is higher than those of the other specifically deuterated phosphocholines. In addition, hydration or solution in chloroform of an anhydrous sample

TABLE I
INFRARED VIBRATIONAL FREQUENCIES OF THE C-D STRETCHING BANDS OF SPECIFICALLY DEUTERATED DPPC AND PALMITIC ACID

Compound	Position labeled*									
	2′-d_2		3′-d_2		7′,8′-d_4		13′-d_2		16′-d_3	
	ν_{as},cm^{-1}	ν_s,cm^{-1}	ν_{as},cm^{-1}	ν_s,cm^{-1}	ν_{as},cm^{-1}	ν_s,cm^{-1}	ν_{as},cm^{-1}	ν_s,cm^{-1}	ν_{as},cm^{-1}	ν_s,cm^{-1}
Palmitic acid solid			2,203.2	2,118.0	2,180.6	2,079.4§	2,169.8	2,094.9	2,221.2 2,210.2	2,073.6
DPPC										
anhydrous	2,219.0								2,216.0	
solid	2,216.0	2,075.0‡	2,196.8	2,112.2	2.180.0	2,078.4	2,169.6	2,095.6	2,212.0	2,074.0
hydrated										
5°C	2,212.6	2,075.1	2,197.6	2,112.8	2,180.2	2,078.9	2,169.7	2,096.2	2,212.2	2,074.6
38°C	2,213.2	2,075.0	2,198.0	2,113.4	2,180.8	2,079.1	2,169.7	2,096.5	2,212.8	2,074.8
50°C	2,213.4	2,075.0	2,200.3	2,115.2	2,184.1	2,082.0	2,173.3	2,099.2	2,213.7	2.075.1

*ν_{as}, frequency of the asymmetric mode; ν_s, frequency of the symmetric mode.
‡In all cases we have listed only the lower, more intense component of the Fermi resonance pair. Weaker bands at about 2,120 cm^{-1} are evident in the spectra of DPPC-2′-d_3, DPPC-7′,8′-d_4, and DPPC-16′-d_3.
§Values are taken from the spectrum of palmitic acid 5,6-d_4. The comparison is valid, however, because in the central region of the acyl chain, frequencies of the CD_2 stretching bands are invariant with position of substitution (this laboratory: unpublished data from specifically deuterated palmitic and stearic acids).

results in a shift to lower frequency of the band, whereas in all other cases both the antisymmetric and symmetric bands shift to higher frequency (Table I). The characteristics of the spectra of hydrated DPPC-2'-d_2 are given in Fig. 1. Fig. 1 *A* shows the C—D stretching region of the infrared spectrum of a fully hydrated multibilayer sample at 5 and 30°C ($T < T_{pre}$), 38°C ($T_{pre} < T < T_m$), and at 45°C ($T > T_m$). Fig. 1 *B* shows a plot of $\Delta A/\Delta T$ vs. temperature for the antisymmetric CD_2 stretching band at about 2,213 cm^{-1} while Fig. 1 *C* and *D* shows, respectively, plots of frequency and bandwidth vs. temperature for the antisymmetric CD_2 stretching band and the symmetric CD_2 stretching component at 2,075 cm^{-1}.

As is shown in Fig. 1 *A*, the main effects resulting from an increase in temperature are decreases in peak heights and changes in the relative heights of the two peaks comprising the antisymmetric CD_2 stretching band.

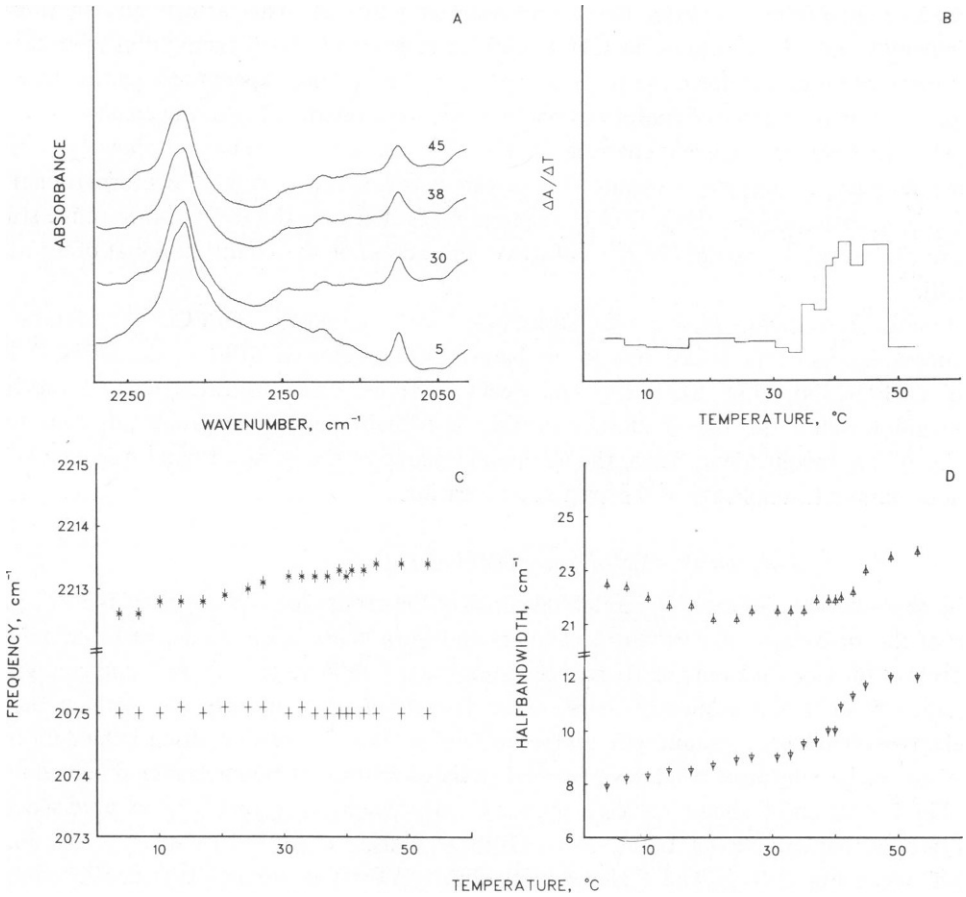

FIGURE 1 Temperature dependence of the CD_2 stretching bands of hydrated bilayers of DPPC-2'-d_2. (*A*) Spectra recorded at 5°, 30°, 38° and 45°C. All spectra are plotted on the same scale. The water association band has been removed by subtraction. (*B*) Plot of $\Delta A/\Delta T$ vs. temperature derived from the antisymmetric CD_2 stretching band near 2,213 cm^{-1}. (*C*) Frequency of the antisymmetric (*) and symmetric (+) band maxima versus temperature. (*D*) Half bandwidth of the antisymmetric (▲) and symmetric (▼) CD_2 stretching bands versus temperature.

The plot of $\Delta A/\Delta T$ vs. temperature, Fig. 1 *B*, shows a broad maximum in the range 35–45°C. As the changes at T_m in a $\Delta A/\Delta T$ plot derived from the CH_2 wagging band progression of the same sample occurred within a 1°C interval, the breadth in Fig. 1 *B* is intrinsic to this location and may indicate that slight changes in this specific part of the bilayer occur over a wider temperature range than is the case in the more hydrophobic regions of the bilayer.

Temperature-induced variations of frequency and bandwidth are minimal. The frequency of the band at 2,075 cm^{-1} is invariant while only a small, monotonic increase in the frequency of the antisymmetric stretching band is evident as the temperature is increased (Fig. 1 *C*). The bandwidths (Fig. 1 *D*) show slightly greater temperature dependencies, with both bands broadening slightly at T_m. However, while the 2,075 cm^{-1} band narrows as the temperature is reduced, a broadening of the antisymmetric band at temperatures below 20°C is evident.

The location of the 2′-CD_2 group adjacent to the polar carbonyl moiety results in a large inductive interaction between the C═O and CD_2 groups. This affects the frequencies, bandwidths, and intensities of the CD_2 stretching bands, and makes them differ from those of groups located further down the acyl chain (7, 12). We can also expect these parameters to be dependent on the degree of conformational disorder and the mobility of the chains.

The temperature-induced changes in the CD_2 stretching bands are, however, minor. Consequently, although we cannot distinguish between the effects of conformational and inductive perturbations, the lack of change indicates that over the temperature range studied there are no major changes in the inductive interaction or the conformational order at this location.

Finally, the antisymmetric CD_2 stretching band contours of DPPC-2′-d_2 contains two principal components, in contrast to the band in the spectra of DPPC-3′-d_2, DPPC-7′,8′-d_4, and DPPC-13′-d_2 (Fig. 2). Seelig and Seelig (13) have demonstrated that in the liquid crystalline phase the two deuterons of the *sn*-2 chain methylene group adjacent to the carbonyl are inequivalent; hence the anomalous shape of the infrared band may result from conformational complexity in this particular location.

DPPC-3′-d_2, DPPC-7′,8′-d_4, and DPPC-13′-d_2

The characteristics of the CD_2 stretching bands of the groups located at positions 3′, 7′, 8′, and 13′ of the *sn*-2 chain of DPPC reflect inter- and intra-chain interactions, with the inductive effects of the ester linkage greatly reduced at position 3′ relative to position 2′ and negligible at position 5′ (12). Consequently, as we have demonstrated in the previous section that the inductive effects are constant with temperature at position 2′, we shall discuss the data in this section solely in terms of the fatty acid acyl chain conformational and motional behavior.

Fig. 2 *A*, *B*, and *C* shows, respectively, the C—D stretching region of the infrared spectra of fully hydrated samples of DPPC-3′-d_2, DPPC-7′,8′-d_4, and DPPC-13′-d_2 at 5, 30, 38, and 45°C while Fig. 2 *D*, *E*, and *F* shows plots of $\Delta A/\Delta T$ versus temperature derived from the antisymmetric CD_2 stretching bands at about 2,190 cm^{-1}.

At temperatures below T_{pre}, the main effect of an increase in temperature on the spectra is to decrease the peak heights. The rates of decrease are small compared to those at T_m, as is shown in the spectra and in the $\Delta A/\Delta T$ plots. At T_{pre} we observe markedly different behavior in the spectra. For DPPC-3′-d_2 and DPPC-13′-d_2 the only effects evident in the temperature

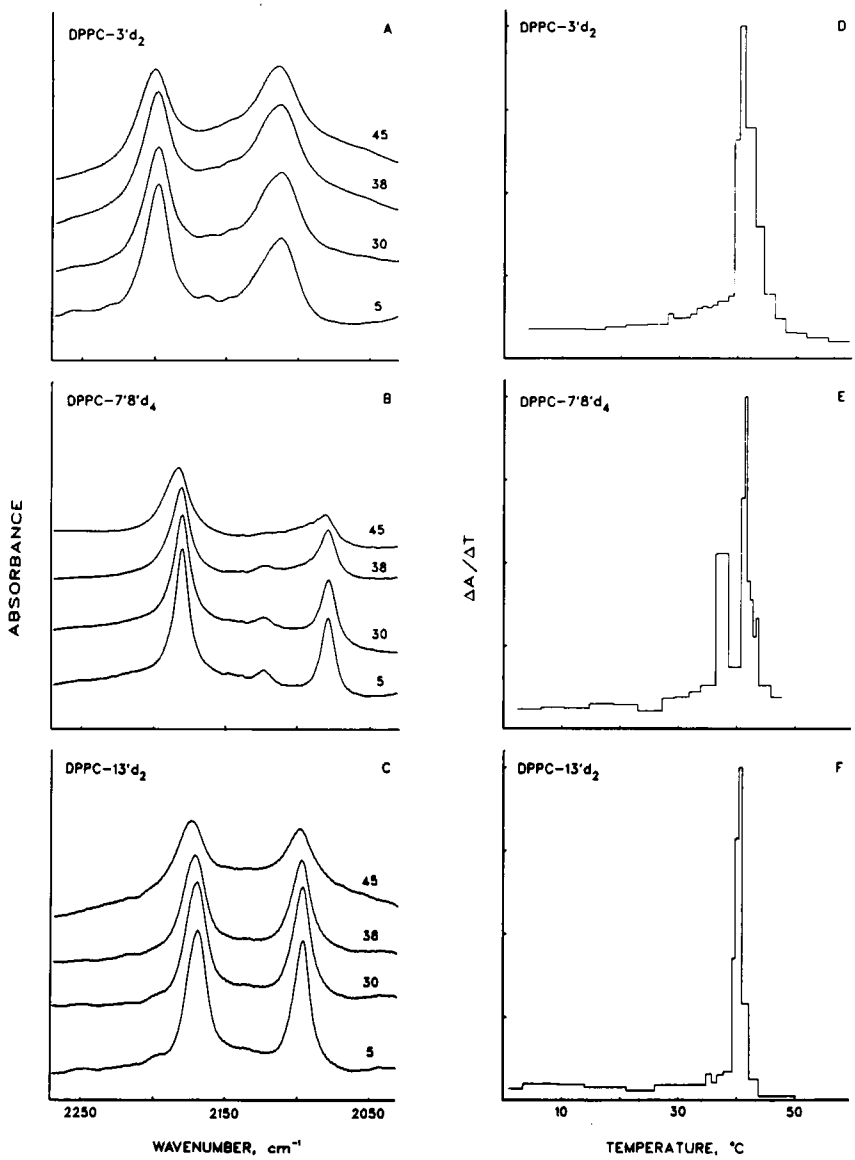

FIGURE 2 (*A–C*) The C—D stretching regions of the infrared spectra of hydrated bilayers of DPPC-3'-d_2 (A); DPPC-7',8'-d_4 (*B*); and DPPC-13'-d_2 (*C*) at 5°, 30°, 38°, and 45°C. The water association band has been removed by spectral subtraction. (*D–F*) $\Delta A/\Delta T$ vs. temperature plots derived from the antisymmetric CD_2 stretching bands of DPPC-3'-d_2 (*D*); DPPC-7',8'-d_4 (*E*); and DPPC-13'-d_2 (*F*).

range 30–40°C are slightly enhanced values of $\Delta A/\Delta T$ (as compared to the values below 30°C), with slight evidence of a weak feature in the $\Delta A/\Delta T$ plot of DPPC-13'-d_2 in the range 34–38°C.

In the spectrum of DPPC-7',8'-d_4, however, the peak heights of the antisymmetric and symmetric CD_2 stretching bands decrease abruptly at T_{pre}, resulting in the large peak in the $\Delta A/\Delta T$ plot in the range 35–37°C.

At T_m the spectra of all three compounds show large changes in all band parameters. These changes occur within a narrow temperature range and result in the strong peaks centered at 41.5°C in the $\Delta A/\Delta T$ plots. Above this temperature, in the liquid crystalline phase, minimal temperature dependencies of the spectra are observed.

We note that in the case of DPPC-3′-d_2, T_m is somewhat broader (Fig. 2 *D*). As mentioned in the experimental section, the CH_2 bands of this sample show sharp transitions; hence, the broadening of T_m at position 3′ is intrinsic to this position. As the broadening of T_m at position 2′ is even greater (Fig. 1 *B*), it would appear to be associated with the hydrophilic region of the bilayer.

A more detailed insight into the thermotropic behavior of the different segments of the acyl chains may be obtained from an analysis of the variations of the frequencies and bandwidths as a function of temperature. These data are presented in Fig. 3, which shows the temperature dependencies of the frequencies and bandwidths of the antisymmetric CD_2 stretching bands of DPPC-3′-d_2 (Fig. 3 *A* and *D*, respectively), DPPC-7′,8′-d_4 (Fig. 3 *B* and *E*, respectively) and DPPC-13′-d_2 (Fig. 3 *C* and *F*, respectively).

As shown in Table I, in all cases the frequencies of the antisymmetric and symmetric CD_2 stretching modes of the dry and hydrated phosphatidylcholines agree to within ± 0.3 cm^{-1}. The frequencies also agree to within ± 0.3 cm^{-1} with those of bands in the spectra of the corresponding specifically deuterated palmitic acids, except for palmitic acid-3-d_2 where the large difference results from the stronger inductive effects of the acid group as compared with the ester linkage of DPPC. Although our technique of bandwidth measurement is optimized for monitoring changes, the values in Fig. 3 are also in substantial agreement (± 4 cm^{-1}) with those of dry films. Examining the temperature dependencies of the frequencies, (Fig. 3 *A*, *B*, *C*, and Table I) one can see that within the temperature range 0–38°C the frequencies of the antisymmetric CD_2 stretching bands are almost invariant. Only slight, steady increases with increasing temperature are observed, the maximum shift being less than 0.6 cm^{-1} in the case of DPPC-7′,8′-d_4. Similar results are evident in the frequency shifts of the symmetric CD_2 stretching bands (Table I). Within the same temperature range the bandwidths increase slightly upon raising the temperature; the rate of increase being marginally higher in the range 30–40°C than at lower temperatures. There is no evidence of any change associated with T_{pre} in either the frequency or the bandwidth plots; spectral changes at T_{pre} are predominantly restricted to the decreases in peak heights in the spectra of DPPC-7′,8′-d_4 (Fig. 2 *B*).

The above observations may be explained as follows. First, as the frequencies of these vibrational modes are sensitive to the introduction of *gauche* conformers (3, 14), and as the frequencies match those of the fatty acids and the anhydrous films, we conclude that in all three systems the acyl chains are in the same conformation. X-ray studies of fatty acids (15) and of dimyristoyl phosphatidylcholine (16) have shown this conformation to be *trans* from position 3′ to the end of the acyl chain. Thus, the acyl chains of DPPC are predominantly *trans* from position 3′ to at least position 14′ (i.e., the position adjacent to the 13′-CD_2 probe), at all temperatures in the gel phase.

Second, slight variations in bandwidths, without concomitant frequency shifts, are indicative of increased rates of motion of the acyl groups as the temperature is increased in the gel

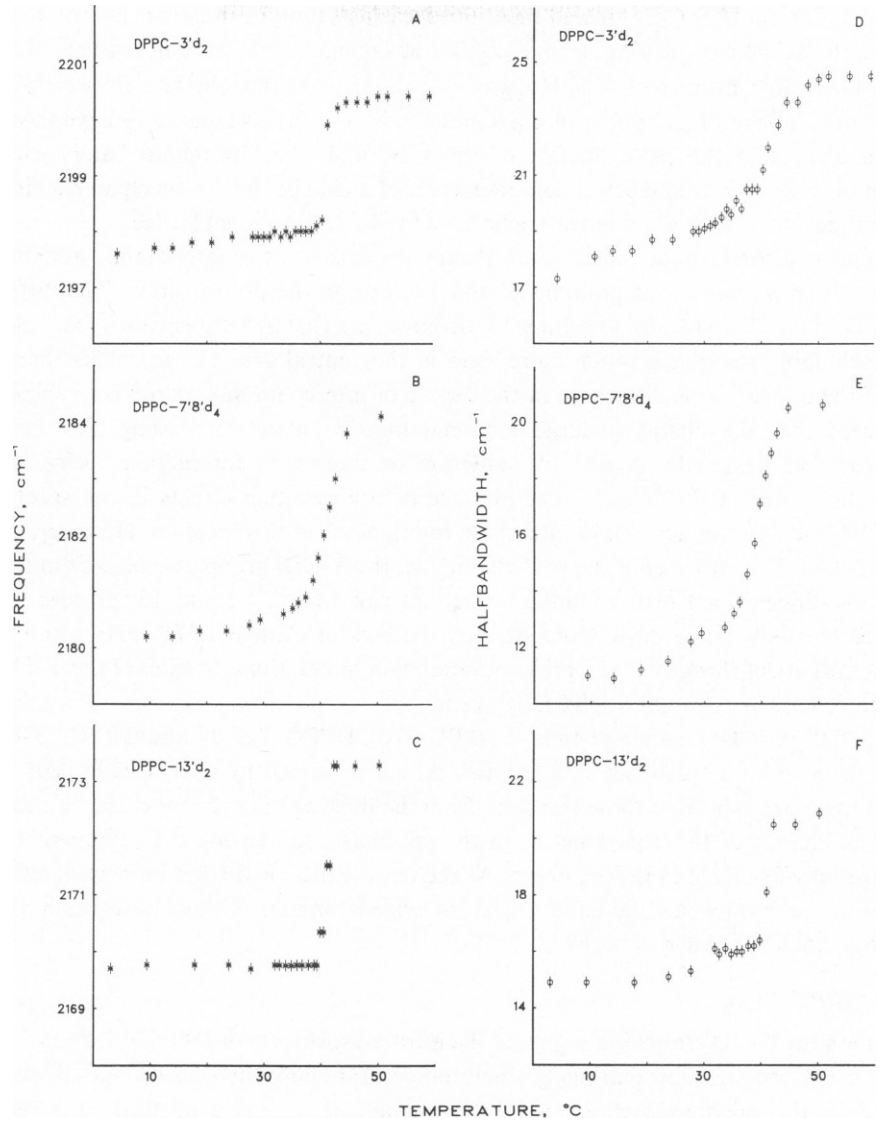

FIGURE 3 (*A–C*) Temperature dependence of the frequencies of the antisymmetric CD_2 stretching bands of DPPC-3'd_2 (*A*); DPPC-7',8'd_4 (*B*); and DPPC-13'-d_2 (*C*). (E–F) Temperature dependence of the bandwidth of the antisymmetric CD_2 stretching bands of DPPC-3'-d_2 (*D*); DPPC-7',8'-d_4 (*E*), and DPPC-13'-d_2 (*F*).

phase. As the chains are *trans*, this must result from librational and torsional motions about the long axes of the acyl chains.

Third, the spectral changes induced by the pretransition are only evident in the central portion of the acyl chains, and result in an abrupt decrease in the peak height with no change in frequency or bandwidth. This behavior is identical with that observed in the CH_2 stretching

modes of DPPC at T_{pre} (3). As was mentioned earlier, the pretransition marks an abrupt change in the acyl chain packing, from a regular hexagonal lattice at temperatures above T_{pre} to an orthorhombic lattice with a high degree of motion about the long axes of the acyl chains at temperatures below T_{pre}. Studies of *n*-alkanes carried out in this laboratory have shown that abrupt decreases in the peak heights of the CH_2 and CD_2 stretching bands without a concomitant change in frequency are characteristic of a change in the acyl chain packing from a rigid lattice, either orthorhombic or triclinic, to the loose hexagonal lattice.

Consequently, these data indicate an abrupt decrease in the interchain interactions at positions 7′ and 8′, but not at positions 3′ and 13′, during the pretransition. The differences between positions 7′ and 8′, and position 13′ demonstrate that at temperatures just below T_{pre} the orthorhombic packing is much more rigid in the central area of the chains than in the center of the bilayer. This difference in the degree of interchain interaction is in accord with the concept that the chains undergo torsional motions about their long axes, with the amplitudes and hence the degree of reduction of interchain interactions being greatest towards the center of the bilayer. The absence of pretransition effects in the spectrum of DPPC-3′-d_2 indicates no change in interchain interactions in this location. However, because of the 2′-3′ *gauche* conformer of the *sn*-2 chain (16), the 3′-CD_2 group cannot pack in the same type of two-dimensional orthorhombic subcell as can the 7′, 8′, and 13′ groups; i.e., one comprised of methylene groups. Consequently the lack of change at T_{pre} may result from a lack of sensitivity of this group as a probe of interchain interactions. Studies of the 4′, 5′, and 6′ deuterated compounds would resolve the question.

At T_m all the C—D stretching bands of DPPC-3′-d_2, DPPC-7′,8′-d_4, and DPPC-13′-d_2 show large changes in both frequency and bandwidth, accompanied by decreases in peak height. These changes are typical of those resulting from the melting of acyl chains and contrast with the lack of change of these parameters in the gel phase, supporting the argument that the chains are fully extended in the gel phase. As the temperature is further increased, only slight increases in the frequencies and bandwidths are evident, similar to those observed in the CH_2 stretching region of spectra of perhydro DPPC.

DPPC-16′-d_3

Fig. 4 shows the C—D stretching region of the infrared spectrum of DPPC-16′-d_3 at 5, 30, 38, and 45°C. The spectra show extremely small temperature dependencies compared with those discussed in the previous section, and little change at T_m, as evidenced in a relatively featureless $\Delta A/\Delta T$ plot (not shown).

Plots of the temperature dependence of the frequency and bandwidth of the asymmetric CD_3 stretching band of DPPC-16′-d_3 are given in Fig. 5 *A* and *B*, respectively. At 5°C the peak maximum is at 2,212.2 cm^{-1}, close to the lower frequency maximum in the spectrum of anhydrous DPPC-16′-d_3. As the temperature is increased the frequency of the band increases, accompanied by a slight broadening of the band. The main transition results in a substantial broadening and increase in frequency, relative to those observed in the gel phase; and constant values are observed above T_m.

In the liquid crystalline phase, the terminal methyl group (4, 14) and the environment in the center of the bilayer are highly disordered. The reduction of both frequency and bandwidth at T_m on lowering the temperature indicates a decrease in the rate of rotation of the

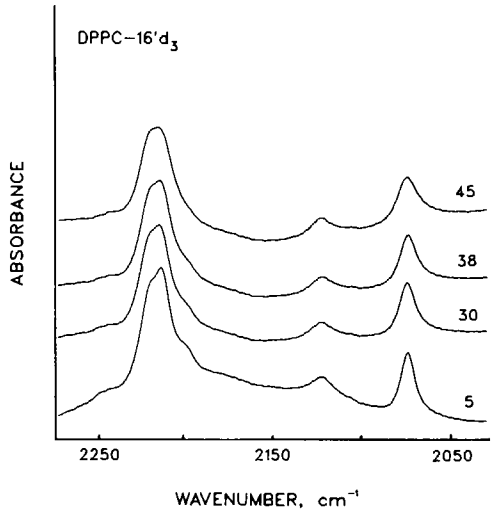

FIGURE 4 C-D stretching region of the infrared spectrum of hydrated DPPC-16'-d_3 bilayers at 5°, 30°, 38° and 45°C. The water association band has been removed by spectral subtraction.

methyl group due to restrictions by the hexagonal packing in the gel phase. The relatively large reduction in frequency at T_m also suggests that the 15'-CH_2 is conformationally ordered in the gel phase, as a high average population of 14'-15' *gauche* bonds is not compatible with a CD_3 spectrum characteristic of a well-packed solid phase.

The factors affecting the methyl group as the temperature is reduced in the gel phase are more complex than those which occur at T_m. This can be demonstrated by examining the data in Fig. 6, which shows the asymmetric CD_3 stretching band of DPPC-16'-d_3 at 5, 20, 30, and 38°C, together with the difference spectra generated by subtraction of adjacent spectra from each other.

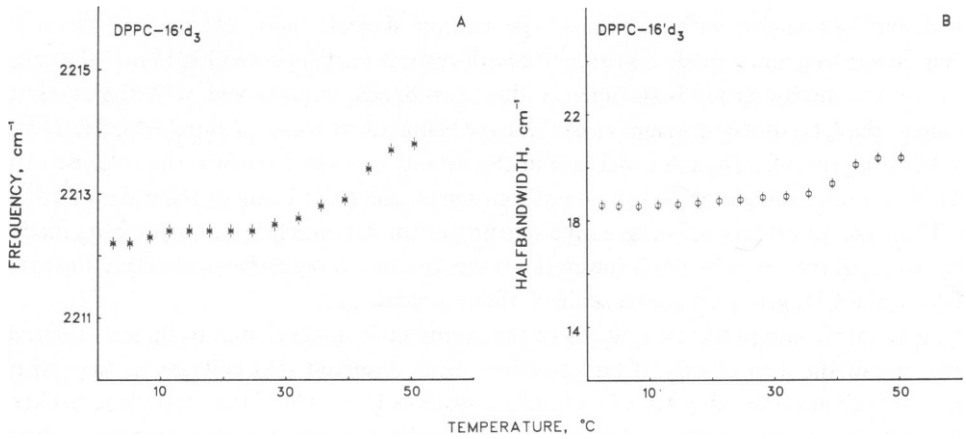

FIGURE 5 Temperature dependence of the asymmetric CD_3 stretching band of DPPC-16'-d_3. (*A*) Frequency vs. temperature. (*B*) Half bandwidth vs. temperature.

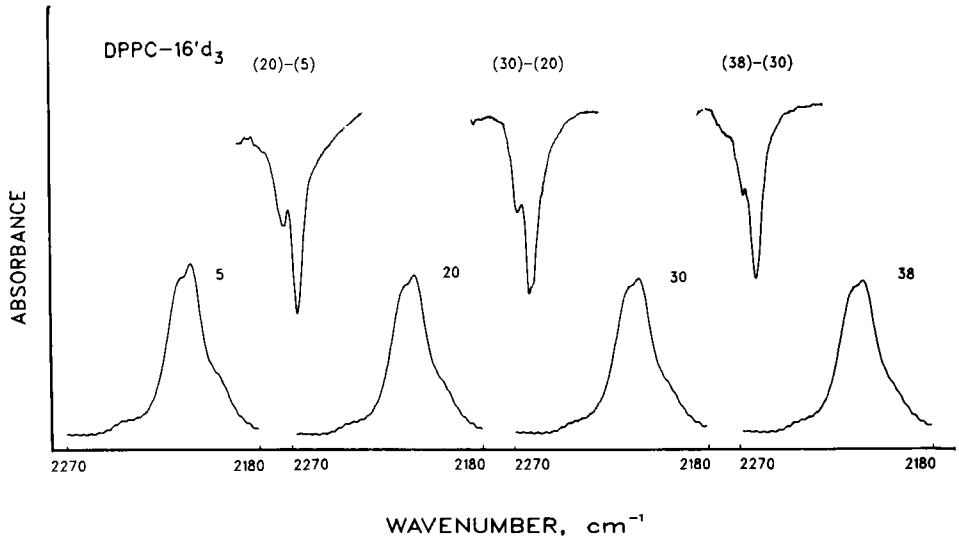

FIGURE 6 Progressive motional collapse of the doubly degenerate asymmetric CD_3 stretching mode of DPPC-16'-d_3. Bottom: Absorption spectra at 5°, 20°, 30°, and 38°C. Top: Difference spectra obtained by subtraction of adjacent pairs of absorbance spectra. Because all spectra are plotted to the same size, absolute values of changes cannot be compared.

At all temperatures in the gel phase the asymmetric CD_3 stretching band is composed of two overlapped peaks. As the temperature is reduced the frequency separation of the peaks increases. This results in the two minima in the difference spectra, even in that obtained by subtraction of the spectra recorded at 30° from that at 38°C. Although the two peaks are not completely resolved at 5°C, the splitting is ~4 cm^{-1}, and further reduction of the temperature to −50°C resulted in a splitting of ~6 cm^{-1} (not shown).

Splitting of the doubly degenerate asymmetric methyl stretching mode is related to the rate of rotation of the methyl group relative to the adjacent methylene group. Two modes are allowed, one symmetric with respect to the carbon skeletal plane of the acyl chain and another, lower frequency mode, asymmetric with respect to this plane (17, 18). If the rate of rotation of the methyl group is sufficiently slow, two bands are observed, with the maximum splitting of the CD_3 mode in a rigid crystal lattice being about 6 cm^{-1} (unpublished data from this laboratory on n-$C_{19}D_{40}$). An increase in the rate of rotation results in the collapse of the band contour into a single peak, the rate of rotation at this point being of the order of 10^{12} s^{-1} (19). Thus, the observation of progressive splitting of the asymmetric CD_3 stretching mode of DPPC-16'-d_3 as the temperature is lowered is indicative of a progressive decrease in the rate of rotation of the CD_3 group on a time scale of about a picosecond.

Being an intrachain property, splitting of the asymmetric mode cannot be directly related to the packing of the acyl chains. It has, however, been observed that collapse of the splitting occurs at much lower temperatures in clathrate systems (19), where the methylene backbone is not constrained by intermolecular interactions and rotates freely, than in neat n-alkanes where the methylene backbone is rigidly constrained (20). The observation of progressive splitting of the asymmetric methyl stretching bands in this temperature range thus provides

an indirect indication of a progressive reduction of the mobility of the acyl chains as the temperature is reduced.

Comparison with Previous Results

The most detailed data regarding the conformations and dynamics of the acyl chains of DPPC in the liquid crystalline phase result from the deuterium NMR studies of Seelig and Seelig (1) and Davis (4). These studies indicate that the degree of conformational disorder is the same in the first ten methylene groups of the palmitoyl chains, and that it then increases towards the terminal methyl groups. This plateau has since been observed in a variety of systems, including natural membranes (2, 21–23), and it has been reproduced by means of a statistical mechanical model of the liquid crystalline phase (24).

The results of the present study confirm that the liquid crystalline phase contains a high population of *gauche* conformers. Because of the limited series of compounds employed, however, we cannot determine whether there is a plateau effect in the infrared spectral parameters.

As was mentioned in the introduction, the only other study of gel phase characteristics of a series of specifically deuterated phosphocholines is that of Bansil et al. (7). They report that immediately below T_m the "disorder" is greatest near the center of the bilayer. As we demonstrate herein that, excepting the 2′-3′ *gauche* bond, the chains are predominantly *trans* in the gel phase, we interpret their data as evidence for increased amplitudes of torsional and librational motions near the terminal methyl group relative to those near the head group. Extensive motion about the long axes of the acyl chains has also been detected by means of saturation transfer electron spin resonance (5) and ^{2}H-NMR (4) techniques.

The latter study also provides data for a direct comparison. Although the ^{2}H-NMR signals from the methylene groups of DPPC-d_{62} are superimposed in the gel phase spectra, the signal from the terminal methyl groups is readily distinguished. Quadrupole splittings of 12.2, 14.9, and 17.3 kHz are observed at 20°, 2°, and −7°C (4). These values may be compared with values of 12.1 and 20.2 kHz of the CD_3 signals of *n*-$C_{19}D_{40}$ in the hexagonal and orthorhombic phases, respectively (unpublished data from this laboratory). It is apparent that as the temperature is reduced the quadrupole splitting progresses steadily from a value near that of the hexagonal phase of *n*-$C_{19}D_{40}$, which exhibits no splitting of the asymmetric CD_3 stretching band in the infrared spectrum, towards the value obtained in the orthorhombic phase, where a 6-cm^{-1} splitting is observed in the infrared spectrum.

The present data may also be considered in relation to other Raman studies of phosphatidylcholines (25–28). The results of these studies have been interpreted as indicating that the acyl chains are all-*trans* at −60°C, but that as the temperature is increased there is a steady increase in the population of *gauche* conformers to a value of 1–4/chain immediately below T_m. We find no evidence of a high population (2–4/chain) of *gauche* conformers in the gel phase, and the exact agreement of the frequencies of all of the CD_2 stretching modes in the spectra of the fatty acids, the anhydrous DPPC films, and the hydrated phosphatidylcholines (Table I) argues against this concept. This is particularly so in the case of DPPC-13′-d_2, as the *gauche* conformers are postulated to be localized near the center of the bilayer (25) and to be introduced in the temperature range 0–38°C. The above conclusions, however, were based on the observation of a decrease in the peak height of the 1,130-cm^{-1} Raman band as the

temperature is increased, and on the presumption that the intensity of this band is linearly dependent on the number of *trans* bonds in the acyl chains. We recently demonstrated (29) that, on transition from the orthorhombic to the hexagonal phase, the 1,130-cm^{-1} band of *n*-C_{21} H_{44} changes by roughly the same amount as does that of DPPC on transition from orthorhombic (−60°C) to hexagonal (38°C) packing. Inasmuch as the population of *gauche* conformers in the hexagonal phase of *n*-$C_{21}H_{44}$ is low (estimates being one or less per chain) the data demonstrate that the 1,130-cm^{-1} band intensity is not linearly related to the number of *trans* conformers. The former point is brought out by Pink et al. (27), who demonstrate a complex relationship between the intensity and number of *gauche* conformers, and who for DPPC estimate 1–1.3 *gauche* conformers/chain in the temperature range 30–40°C.

With these lower estimates, ~1/chain, the infrared and Raman data are in general accord. The resolution beyond this point would require an extremely detailed study of selectively deuterated compounds using a combination of ^{2}H-NMR, Raman, and infrared spectroscopy.

SUMMARY

We have presented the results of a detailed study of the thermotropic behavior of the C—D stretching bands of a series of specifically deuterated DPPC. The spectra obtained at temperatures below T_m indicate that throughout the gel phase the acyl chains are extended and contain no substantial average population of *gauche* conformers. Spectral changes at T_{pre} have been previously shown to result from changes in the interchain interactions, but in this study the effects of changes in the interchain interaction are only evident in the spectra of DPPC-7′,8′-d_4. This supports the concept of a "loose" orthorhombic subcell below T_{pre}, with the amplitudes of librational and torsional motions about the long axes of the acyl chains being greatest near the center of the bilayer. There may, in fact, be a plateau effect with the interchain interactions dropping off rapidly near the center, but a more complete series would be required to confirm this postulate.

As the temperature is reduced below T_{pre}, changes in the CD_2 stretching bands are minimal. Progressive splitting of the CD_3 bands is observed, however, and is indicative of increasingly rigid orthorhombic packing.

The main melting transition, at T_m, results in changes in all the C—D stretching bands monitored. Changes in the spectra of DPPC-2′-d_2 are small and show that there is no large change in the inductive interaction of the ester linkage with the acyl chain. Changes in the C—D stretching bands of DPPC-3′-d_2, DPPC-7′,8′-d_4, and DPPC-13′-d_2 are indicative of the introduction of large numbers of *gauche* conformers, whereas the changes in the spectrum of DPPC-16′-d_3 result from an increase in the rate of rotation of the methyl group and the overall increased mobility in the liquid-crystalline phase.

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